THE INFLUENCE OF HYDROGEN ION CONCENTRATION ON UNFERTILIZED *ARBACIA, ASTERIAS* AND *CHÆTOPTERUS* EGGS.

HOMER W. SMITH AND G. H. A. CLOWES.

(From the Lilly Research Laboratory, Indianapolis and the Marine Biological Laboratory, Woods Hole.)

In a previous paper the writers briefly reported experiments on the effects of increased H-ion concentration in sea water on the fertilization and development of the normally fertilized eggs of the star fish (*Asterias forbesii*) and sea urchin (*Arbacia punctulata*) (1). This work has since been repeated and extended in several directions and more complete reports on these subjects are now ready for publication. The experiments to be reported in this paper deal with effects of acid and alkaline sea water on the rate of ageing of unfertilized *Arbacia, Asterias* and *Chætopterus* eggs, and with the artificial activation of *Chætopterus* eggs by acid sea water. These experiments, beside affording a necessary basis for further studies of the influence of variations in H-ion concentration on the fertilization and developmental processes, furnish important information on the relation of the physiological activity of the egg cell to its environment.

Preparation of Acid and Alkaline Sea Water.

Certain of our experiments, which will be described in a subsequent paper, have shown that CO_2 exerts a profound effect, distinguishable from the effects of H ions, on many of the physiological processes of marine eggs. Since sea water naturally contains a considerable quantity of combined carbonic acid, it is necessary in all experiments designed to observe the effects of H ions *per se* to work with sea water from which the CO_2 has been removed. The CO_2-free sea water used in these experiments was prepared as follows: To each liter of fresh sea water was added 5 cc. of $N/2$ HCl and 5 cc. of $N/10$ NaH_2PO_4; this mixture was aërated with a water vacuum pump over night. No pro-

vision was made for excluding the CO_2 of the atmosphere because the concentration in equilibrium with the atmosphere after aëration is, so far as our experiments are concerned, negligibly small. $N/40$ NaOH was used for restoring this acid sea water to the desired H-ion concentration, using colorimetric standards with a salt content equivalent to that of sea water prepared as recommended by Clark (2) and McClendon (3). NaH_2PO_4 was added to the acidified sea water to give it greater buffering capacity in the neighborhood of neutrality, a rôle normally played by $NaHCO_3$. A sufficient quantity of acid sea water was made fresh for each day's use, and the individual solutions were prepared immediately before using.

CO_2-free sea water prepared in this manner was used between pH 4.5 and 8.0. In solutions more alkaline than pH 8.0 basic phosphates are thrown out, so solutions more alkaline than sea water were prepared by adding $N/10$ NaOH to natural sea water. In this case the carbonates do not interfere because the CO_2 tension is negligibly small. At pH 10.2 $Mg(OH)_2$ begins to precipitate; the amount of alkali required to complete this precipitation is many times the amount required to bring sea water from its normal reaction to 10.2. If the precipitation of $Mg(OH)_2$ were completed, the resulting solution would be physiologically unbalanced. It is, therefore, impossible to go beyond this point on the alkaline side. Consequently our observations are limited to the range pH 4.5 to 10.2, which embraces all the physiological variations with which we are concerned.

The addition of HCl and NaOH dilutes the salt content of the sea water slightly but we have no reason to believe that this slight degree of hypotonicity has introduced any serious complication into our results. Nearly all experiments reported in this paper were performed at approximately 20° C. Early in the season it was necessary to bring the sea water to this temperature, but later it was easily maintained with a slight regulation of the temperature of the laboratory.

The Viability of *Arbacia* and *Asterias* Eggs.

Asterias eggs were left in sea water until the maturation process was practically completed, during which time they were

washed and gently agitated several times. *Arbacia* eggs were washed with sea water twice after removal from the ovaries and then used at once. The eggs were gathered into a concentrated suspension by gently centrifuging and a few drops of this suspension added to 200 cc. of the pH solutions contained in finger bowls. A small quantity of eggs was used so that there would be little crowding when the eggs settled to the bottoms of the bowls. During the exposure they were frequently agitated. At various intervals samples were transferred to sea water and inseminated with fresh sperm. The samples were examined after the eggs had had time to divide, noting both the number which fertilized, as shown by the presence of a fertilization membrane, and the number which had divided.

The results of several experiments with each species have shown that, as might be expected, there is some variation in the behavior of eggs from different individuals. The extremely artificial method by which the eggs are obtained—that is the excision of the ovaries and the consequent forced shedding—is not a method which could be expected to give eggs of uniform physiological quality from several individuals. But differences in the actual time of survival of eggs from different individuals, though important in some respects, do not materially alter the relative time of survival at different H-ion concentrations. Since the actual time of survival in any one experiment has no particular significance, it has been thought best to omit the extensive tabular data and to express the results in diagrammatic form with such quantitative expression as can reasonably be implied.

The relations between viability and H-ion concentration of *Arbacia* and *Asterias* eggs are given in Figs. 1 and 2. In these figures each contour line represents the proportion of eggs surviving at a definite interval after transfer to the pH solutions, the time of testing being marked on each curve. The data are based on the average results obtained from several experiments. The dotted curves marked "cytolyzed" show the proportion of cytolyzed eggs at the conclusion of the experiments. The full ordinate in these and all following diagrams indicates the H-ion concentration of sea water, *i.e.*, pH 8.15.

It might be supposed that marine eggs would retain for the

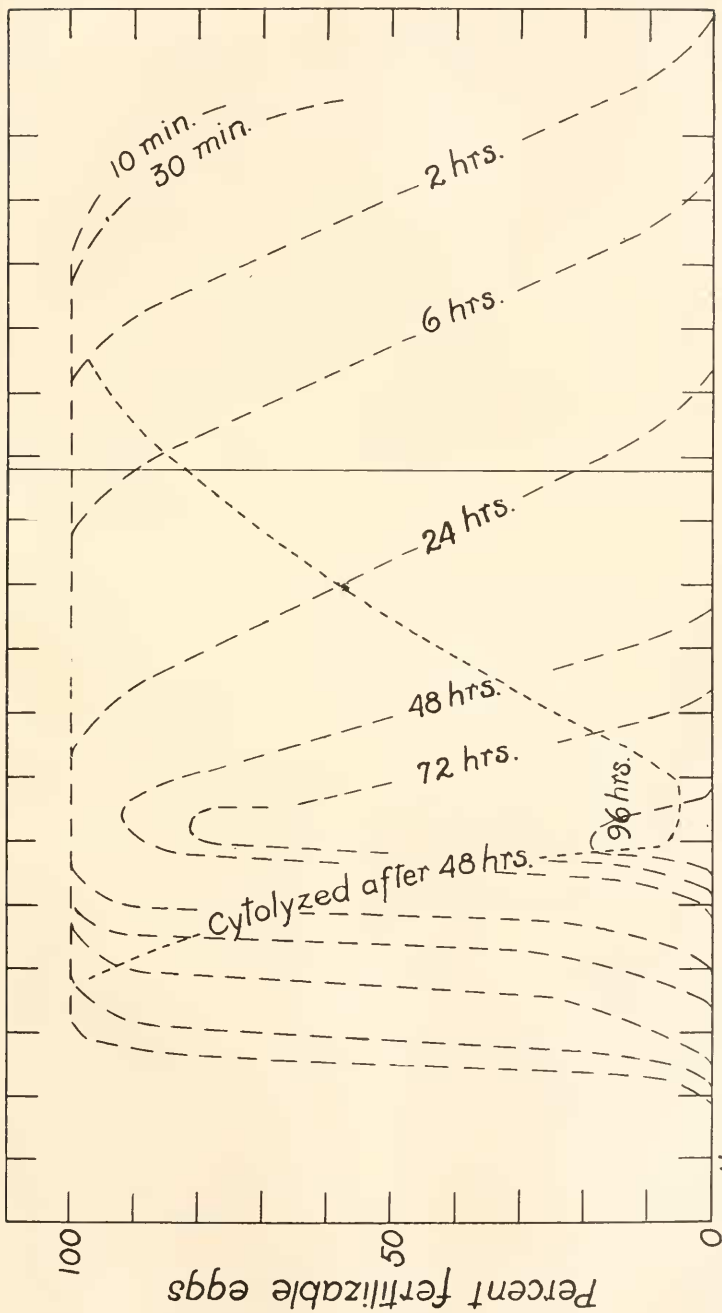

FIG. 1. The proportion of *Arbacia* eggs surviving after the stated exposures to acid and alkaline sea water.

longest time their capacity to fertilize (or to develop) in their normal habitat, sea water, the H-ion concentration of which rarely varies beyond pH 7.8 to 8.4. Contrarily, both *Arbacia* and *Asterias* eggs retain their viability longest, and are least susceptible to cytolysis, in quite acid solutions; the optimum reaction for *Asterias* lies between pH 6.2 and 6.6, and for *Arbacia,* 5.8 to 6.0. The eggs of both species die with great rapidity in very alkaline (pH 10.0) and very acid (pH 4.5) solutions; the rate of injury appears to decrease uniformly as the optimum is approached, particularly from the alkaline side. There is no disproportionate alteration in the rate of death around the pH of sea water. It will be noted that the optimum does not occur midway between the extremes but nearer the acid limits. That is, with respect to the optimum, the eggs of both species can tolerate a larger increase in alkalinity than in acidity. The *Arbacia* egg retains its fertilizing capacity much longer at all H-ion concentrations than does the *Asterias* egg.

It is well known that batches of *Asterias* eggs are frequently encountered in which all the eggs will not maturate or, even when maturated, will not fertilize. Ralph Lillie (4) has shown that the general physiological condition of such eggs could be greatly improved by treatment with ether, as shown by an increase in the number of eggs which both fertilize and divide. Similarly we find that after a short exposure to slightly alkaline sea water (pH 8.2 to 9.4), the proportion of fertilized eggs in these refractive lots is increased. This is expressed in Fig. 2 by the slightly higher incidence of fertilization on the alkaline side of sea water. This effect is clearly a more or less permanent alteration of the egg, since the eggs were transferred from the alkaline solution to normal sea water before insemination. It has sometimes been observed that the proportion of fertilizable eggs is decreased by a short exposure (3 to 5 minutes) to pH 10.0–10.2, and subsequently increased by longer exposures, short of permanent injury. No reason can be given for this apparent transient injury.

Goldfarb (5) has made careful studies of the consequences of ageing in sea water of three species of sea urchin eggs. *Arbacia, Hipponoë* and *Toxopneustes.* He finds that with increased ageing there is an increased tendency for agglutination, fusion of

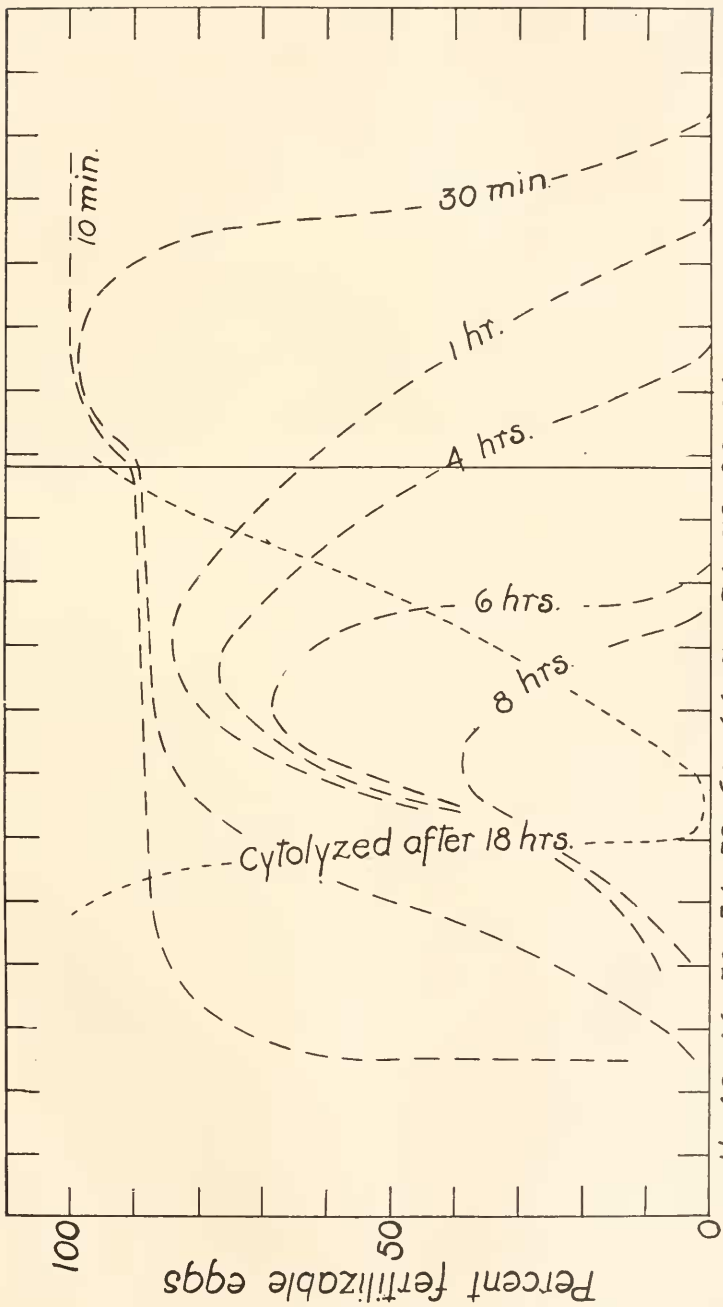

FIG. 2. The proportion of *Asterias* eggs surviving after the stated exposures to acid and alkaline sea water.

eggs and blastulæ, irregular cleavage as manifested in change of size and shape of the blastomeres, retardation in the rate of cleavage, and in extreme stages a total loss of the capacity to cleave. There is also an increase in volume and a loss of the jelly normally surrounding the fresh egg; in *Toxopneustes* and *Hipponoë* there is an initial acceleration in the rate of membrane formation, and in all three species a subsequent retardation of this rate and ultimately a complete loss of the capacity to lift a membrane. Goldfarb attributed the changes accompanying ageing principally to changes in the cortical layer, which, he states, are in turn referable to changed metabolism.

Our results confirm Goldfarb's findings in respect to the loss in *Arbacia* of the capacity to divide. This is equally true at all H-ion concentrations. In *Asterias,* however, on the alkaline side of the optimum the capacity to divide is lost before the capacity for membrane formation. But on the acid side of the optimum (pH 6.2 to 6.5) practically all eggs which lift membranes develop through the first few cleavages.

In both species the tendency for polyspermy increases in proportion to the physiological, rather than the temporal, age of the eggs. Consequently, the incidence of polyspermy is decreased at the optimum to about the same extent to which the viability of the eggs is increased. It is difficult to distinguish polyspermic from abnormally dividing eggs without cytological examination, and therefore it is deemed inadvisable to draw conclusions from our data concerning the tendency for polyspermy after ageing at various H-ion concentrations. It may be said, however, that among those eggs which are aged from pH 6.2 to 6.5 there is a decidedly lower incidence of both definite polyspermy and irregular division, as contrasted with eggs which are aged in more alkaline solutions. The former, if they divide at all, tend to divide regularly through at least two or three cleavages.

The ageing of *Arbacia* and *Asterias* eggs in sea water is accompanied by a slight increase in volume and fluidity. The nucleus which is difficultly discernible in the fresh mature egg, appears in the stale egg as a distinctly defined, hyaline vesicle near the center of the egg. Later, when the egg loses its fertilizing capacity, the cytoplasm becomes distinctly granular and opaque and

the even contour of the egg is lost. As Chambers (6) has shown by microdissection, the granules in the dead egg are disintegrative products and not comparable to the granules in the living egg. They are no longer glutinous or adhesive; the egg has entirely lost its original homogeneity and is held together only by the investing vitelline membrane. Gradually this disintegrative mass imbibes water and swells within the vitelline membrane, becoming a more or less vacuolated liquid mass. Ultimately, the membrane breaks and the contents are dissipated in the sea water.

When *Asterias* eggs are allowed to age in acid and alkaline sea water, the transformation of the nucleus and the subsequent granulation of the cytoplasm occurs most rapidly in solutions more alkaline than sea water, and at about the same rate from pH 8.0 to 5.4. At acidities greater than pH 5.4 the nuclear transformation is perceptibly retarded and the cytoplasm acquires a granular appearance which differs from that of eggs aged in more alkaline solution principally by a diminished degree of discoloration.

From pH 5.4 to 6.2 many eggs are observed which contain, instead of a single vesicular nucleus, two, three or more smaller contiguous vesicles. Such eggs are observed much less frequently in solutions more alkaline than the optimum, pH 6.2. These polyvesiculated eggs will, when returned to sea water and inseminated, lift normal, turgid fertilization membranes in 3 to 5 minutes, and will usually cleave simultaneously into several blastomeres. If not inseminated when returned to sea water, a very small per cent. of the polyvesiculated eggs will fragment once or twice, the vesicles apparently being distributed among the fragments. Although the process of migration of these vesicles into the fragments prior to cleaving was not observed, they appear to be causally related to the process of fragmentation. Fertilization membranes are not formed spontaneously on the polyvesiculated eggs either in the acid by exposures of two to three hours, or when returned to sea water; though a few eggs will form fertilization membranes if left in the acid solutions for considerably longer periods, 4 to 6 hours. The fragments are held together by a delicate membrane bridging the furrows; this may be the vitelline membrane of the unfertilized egg.

The nucleus of the *Arbacia* egg acquires a vesicular appearance

on ageing much the same as that of the *Asterias* egg. As in the latter case this change occurs in about the same time at all H-ion concentrations from pH 5.0 to 10.0. At acidities greater than pH 5.0 there is an increased incidence of eggs in which this nuclear change does not take place. We have not observed the appearance of several vesicles in the *Arbacia* egg at any H-ion concentration, but our observations would not preclude their existence.

In solutions more alkaline than pH 9.0 there is a tendency for membranes to lift spontaneously on both *Asterias* and *Arbacia* eggs. Prior to membrane formation the cortex of the egg undergoes peripheral disintegration with formation of droplets. Spontaneous membrane formation decreases with increasing acidity; below pH 7.0 it is rarely observed except when induced in *Asterias* eggs by long exposures to pH 5.4–6.2, as mentioned above.

Changes in Physical Properties.

The appearance of eggs which have been exposed for a short time to extremely acid or alkaline sea water is markedly different. Alkali treated eggs present a smooth, almost glassy surface, while acid treated eggs are dull and appear to have a finely granulated surface. The slightest amount of manipulation indicates that the alkali treated eggs are soft and more liquid than normal, while eggs treated with acid are more solid. Dr. Chambers has kindly examined the effects of acid and alkali on these eggs by means of microdissection. He finds that in acid sea water the thin, delicate vitelline membrane which normally surrounds the unfertilized *Arbacia* and *Asterias* egg is toughened so that it is difficult to tear. This toughened membrane makes it difficult to ascertain mechanically what influence the acid solution may have on the consistency of the egg surface itself, but with non-injurious exposures acid seems to produce no very profound change in the consistency of the egg cortex. Longer exposures lead to a gradual setting or gelation of the cortex (and possibly the egg as a whole), finally accompanied by the loss of its normal transparency.

In alkali both *Asterias* and *Arbacia* eggs are rendered extremely plastic, soft and liquid by short treatment. This can be shown by shaking the eggs for a uniform time in solutions of increasing alkalinity. Thus when *Asterias* eggs are vigorously shaken in sea

water of pH 5.0 to 10.2 after an exposure of 10 minutes, very little cytolysis or fragmentation occurs between pH 5.0 and 9.0. At pH 9.3 there is a slight amount of fragmentation and a slightly increased number of cytolyzed eggs. A large number of the eggs are distorted from their normally spherical shape, showing that they have softened. At pH 9.6 a few eggs are broken into smaller fragments and the number of intact but cytolyzed eggs is diminished as compared with 9.3. At pH 9.9 the membrane and egg cortex are abruptly destabilized and all the eggs are readily broken into small, spherical and extremely stable fragments.

Similarly the *Arbacia* egg is comparatively resistant to moderate shaking between pH 5.8 and 9.3. At about pH 9.6 there is a marked increase in the tendency to cytolyze. This egg does not fragment as does the *Asterias* egg, presumably because of its inability to form new surface films readily, but appears to cytolyze rather slowly after rupture at some one point. From pH 9.6 to 10.2 the shaken suspensions are filled with ghosts and partially cytolyzed eggs.

The Maturation of *Asterias* Eggs.

The maturation of *Asterias* eggs is normally initiated as soon as they are removed from the ovaries and come in contact with sea water. The initiating factor or factors are not known. Loeb (7) has shown, however, that the addition of acid to sea water inhibits, and the addition of alkali favors the maturation process. When slowed below a critical velocity the maturation process stops and the eggs remain permanently immature.

In view of the possible rôle of H- or OH-ions in initiating maturation, an examination was made of the effects of increasing acidity on the incidence of permanently immature eggs. The eggs were introduced into the pH solutions without contact with sea water by dipping small pieces of fresh ovary into the pH solutions. After 45 minutes or an hour counts were made of the mature and immature eggs, discriminating by the dissolution of the wall of the germinal vesicle. A summary of experiments of this nature is given in Table I.

TABLE I.

NINE EXPERIMENTS ON THE INFLUENCE OF H-ION CONCENTRATION ON MATURATION OF *Asterias* EGGS.

Per Cent. Permanently Immature.

pH									
6.0	6.2	6.4	6.6	6.8	6.9	7.0	7.2	7.4	8.15
	91	72	58	32	28	45	30	6	0
	88	20	44	40	23	40	12	4	0
99	90	82	43	26	8	27	5	1	0
	58	34	22	16	8	18	10	4	0
	76	80	68	63	48	50	35	31	0
	93	76	69	46	60	64	25	10	1
95	88	71	61	39	36	26	14	5	8
93	86	68	69	57	33	16	17	5	1
	76	20	28	33	39	21	17	4	5

Maturation is practically inhibited at pH 6.0, a point at which normally fertilized *Asterias* eggs will grow quite normally, and approximately the point at which the unfertilized egg retains its viability for the longest time. It should be noted in Table I. that the incidence of maturated eggs does not always increase uniformly with increasing alkalinity, but that in some experiments the proportion of immature eggs falls to a low value on the acid side of neutrality, rises noticeably at pH 7.0 (or 6.9) and then falls to zero at 8.15. This irregularity in the influence of H-ion concentration on the maturation process is not affected by washing several times in the respective pH solutions. It appears, therefore, to be attributable to alterations in the egg cortex rather than to the activity of some substance in the supernatant fluid.

THE INFLUENCE OF H–ION CONCENTRATION ON *Chætopterus* EGGS.

The *Chætopterus* egg differs from echinoderm eggs in that it is activated by sea water to which HCl has been added, as Loeb showed many years ago. This activation, though not qualitatively nor quantitatively sufficient to produce normal larvæ, makes it necessary to consider separately the consequences of exposing the unfertilized egg to acid solutions, and the effects of such exposures on subsequent fertilizability.

Like the egg of *Asterias,* the *Chætopterus* egg is shed immature; though the germinal vesicle breaks down when the egg is placed in

sea water, maturation proceeds only as far as the formation of the first maturation spindle. Unless the egg is fertilized or artificially stimulated it remains in the metaphase of the first maturation division. After fertilization the maturation divisions are completed and the polar bodies are formed at the animal pole. Mead (8) first called attention to the fact that a small amount of KCl added to sea water causes these eggs to complete the maturation process with polar body extrusion, an event in this case signifying initiation of development. Three years later Loeb (9), having succeeded in obtaining parthenogenetic development of echinoderm eggs by the use of hypertonic sea water, tried similar procedures on *Chætopterus* eggs. He also examined the influence of KCl and concluded that activation in this case was not a consequence of increase in osmotic pressure, but a specific effect of K-ions. In a careful description of the parthenogenetic development of this egg, he pointed out that after treatment with KCl, development and differentiation appear to proceed without cell cleavage (an observation subsequently confirmed by F. Lillie, 10), and that activation of the egg is accompanied by marked ameboid movements of the protoplasm. He did not succeed at this time in obtaining development with cleavage, though later in collaboration with Wasteneys he obtained cleavage by the combined use of $SrCl_2$ and ox serum. Loeb also decribed a marked tendency in *Chætopterus* eggs activated by KCl to adhere to each other; as a result of this adhesion, larvæ might be formed consisting of several swimmers partially fused, or in some instances, of giant swimmers resulting from the complete fusion of several eggs.

Loeb also obtained ciliated, unsegmented larvæ by treating *Chætopterus* eggs with 100 cc. sea water + 2 cc. $N/10$ KOH, and with 100 cc. sea water + 2 cc. $N/10$ HCl. He mentions the fact of activation by HCl as striking since he had failed to get activation of echinoderm eggs by similar treatment. (It was not until five years later that he tried the fatty acids with signal success.) Allyn (11) has recently examined the action of several acids on *Chætopterus* eggs. She failed to get segmentation and concluded that acids are less effective than KCl.

In our experiments with *Chætopterus* we have observed the

effect of acid and alkaline sea water both in respect to activation and to the subsequent fertilizability after varying exposures. The worms were removed from their tubes as soon as they were brought into the laboratory, and the males and females were placed in separate dishes with running sea water. Before use the females were rinsed well with tap water, then with sea water, and placed in about 50 cc. of sea water in a finger bowl. The egg sacks were cut and the ovaries removed and gently teased apart. After about 15 minutes when all the ripe eggs were shed, the tissue fragments were picked out, the egg suspension filtered through cheese cloth and the eggs concentrated by centrifuging. Because of the small quantity of eggs available, it was necessary to reduce the volume of the pH solutions to 50 cc. Equal quantities of maturated eggs were added to each of the pH solutions; at various intervals portions of these eggs were transferred to two dishes of sea water, only one of which was inseminated. Fresh sperm from one male were obtained when desired by cutting a new sperm sack and allowing the escaping sperm to accumulate in a small quantity of sea water.

The activation of *Chætopterus* eggs by H-ions is illustrated in Fig. 3 by the dotted line. The activation is most intense at pH 5.8 and diminishes rapidly on either side of this point, practically disappearing at 5.0 and 6.6. If the eggs are left at pH 5.8 polar bodies are extruded in 30 to 50 minutes, and about 50 per cent. or more will at the end of two or three hours show marked ameboid movement and extensive fragmentation by budding. The egg flows spontaneously into several unsymmetrical pseudopodia and these in turn develop smaller extrusions, many of which bud off into small spherical fragments. Nuclear division apparently does not always precede this fragmentation, which seems to be largely a result of the ameboid activity of the cortex. If, however, the eggs are exposed for one or two hours to the acid solution and then returned to sea water, 50 per cent. or more will undergo one or two segmentations which more or less simulate the divisions of the normally fertilized egg. They do not develop beyond the two- or four-cell stage, however, and in the majority of instances the cleavages are irregular and the blastomeres tend to separate. If the eggs are returned to sea water after shorter ex-

posures a still smaller proportion of them show signs of activation. A 30-minute exposure will produce polar body formation but only a few eggs will fragment. A 10-minute exposure is insufficient to activate at any H-ion concentration; attention is called to this point because this exposure produces changes in the egg which prevent fertilization by sperm.

There is also a slow activation in alkaline sea water, beginning about pH 9.6 and increasing to 10.2. The activation is apparently not so intense as at pH 5.8 since an exposure of 4 to 5 hours is required to induce segmentation.

It should be emphasized that the H-ion activation of *Chætopterus* eggs is not strictly comparable to the activation of echinoderm eggs by the fatty acids. Loeb showed that in *Arbacia* the strong acids were practically incapable of activating; the fatty acids are efficient by virtue of their penetrating power. Loeb obtained slight activation of *Asterias* eggs by treatment with sea water acidified with HCl (7), but we have obtained no activation of either *Asterias* or *Arbacia* eggs by CO_2-free sea water as acid as pH 4.5. It is probable that the activation obtained by Loeb was due to free CO_2, which Delage has shown to be an excellent activating agent (12).

If *Chætopterus* eggs which have been exposed to the pH solutions for 10 minutes are returned to sea water and inseminated, those from solutions in the neighborhood of pH 5.8 will not fertilize; they remain inert when the eggs taken from the solutions at pH 4.6 or 7.6 have fertilized and have undergone two or 3 normal cleavages. A 10-minute exposure to these solutions, though insufficient to activate, apparently produces some block to fertilization which is not produced by equal exposures to pH 4.6 or 7.6, or to the alkaline solutions which also activate. (The solid line in Fig. 3 shows the proportion of eggs which fertilized in sea water after an exposure of 10 minutes to the pH solutions.) But if the eggs are left in the pH solutions for 30 minutes or longer before being transferred to sea water and inseminated, they slowly recover their fertilizability, in as much as the addition of sperm causes them to segment normally to the 16- or 32-cell stage, and to develop into swimming larvæ of more or less normal appearance. The longer the exposure to the pH solutions, the greater the pro-

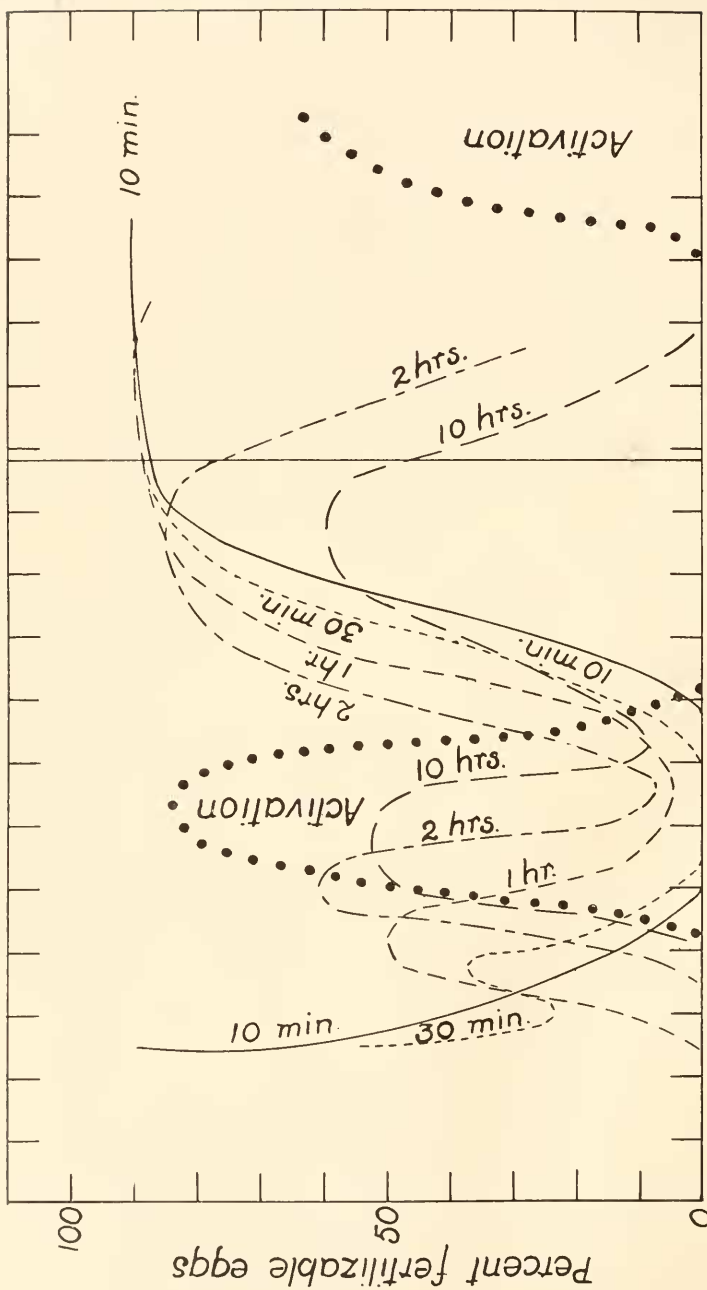

FIG. 3. The heavily dotted lines show the regions of artificial activation of *Chaetopterus* eggs by acid and alkaline sea water. The solid and the dashed lines show the proportion of eggs which fertilize when returned to sea water and inseminated after the stated exposures to the pH solutions. A block to fertilization is created by short exposures to those solutions having the greatest activating power; when the eggs are left in these solutions this blockgradually disappears.

portion of eggs which recover their fertilizability. (The dashed contour lines in Fig. 3 show the general course of this recovery process. The figures written beside each curve show the duration of the exposure to the pH solutions.) The block to fertilization produced by short exposures to the solutions around pH 5.8 disappears only when the eggs are left in the acid solutions. If returned to sea water after a short exposure (*i.e.*, 10 to 30 minutes) the eggs remain permanently (4 to 6 hours) unfertilizable.

Dr. Chambers has examined these eggs by microdissection and finds that when treated for 5 minutes at pH 5.8 and then placed in sea water, the membrane which envelops both the fertilized and unfertilized egg is very much thickened and toughened. Longer exposure to pH 5.8 tends to soften this membrane so that when returned to sea water it is thin, delicate and easily torn. Though the conclusion is by no means substantiated, it is possible that this initial toughening of the membrane with subsequent softening on longer exposure accounts for the inability of sperm to react with short exposure as compared to long exposure eggs.

That the sperm gain access to the egg after the block has worn off is shown by the fact that without sperm they undergo at the most two or three cleavages which are decidedly late and irregular, while with sperm they develop with much more normal velocity and with a quality that is so nearly normal that in many instances they cannot be distinguished from normally fertilized eggs. Many of them, moreover, develop into rough swimmers. There is a marked tendency for the blastomeres to fuse in the later stages with the production of syncytia; and for the separate blastulæ to fuse, 4 or 5 forming one large, apparently homogeneous larva, or for several to adhere together forming irregular chains. This tendency for fusion, like the ameboid movements which accompany activation and normal division, is clearly a consequence of some lability of the cortex. Fusion appears to be more marked among those eggs exposed to solutions on the acid side of pH 5.8 than on the alkaline side.

If we neglect the temporarily irreversible block created at pH 5.8, the optimum reaction for the retention of viability will probably be somewhere in this neighborhood, *i.e.*, pH 5.8 to 6.0. It is certain that this optimum is considerably on the acid side of sea

water, as is the case in *Arbacia* and *Asterias,* and comparatively close to the limits of acid tolerance.

The scarcity of material made it impossible to examine more closely the influence of reaction on the physical properties of the *Chætopterus* egg. It may be stated, however, that unlike *Asterias* and *Arbacia,* the *Chætopterus* egg is distinctly liquified rather than solidified at pH 4.6 to 5.0. This liquefaction is so marked that after an exposure of one or two hours the eggs are extremely fluid and will flow into thin pencils when the containing vessel is tilted or jarred. A similar liquefaction takes place from pH 9.0 to 10.0.

There is no doubt that physical changes in the nature of coagulation in acid and dispersion in alkali characterize the limits of physiological tolerance in *Arbacia* and *Asterias* eggs. But that coagulation in acid is not the invariable rule is evident from the liquefaction which occurs in *Chætopterus* eggs. It is reasonable to suppose that the specific composition of the cortex (and perhaps of the vitelline membrane as well) determines both the direction and degree of the physical changes at various reactions. The cytoplasm of all the eggs examined here is distinctly liquid (13) and it may be that the liquefaction or gelation observed is more a consequence of changes in the vitelline membrane and cortex than in the cytoplasm proper.

The general nature of the changes in physical properties and the changes accompanying ageing at various H-ion concentrations suggests that the prolongation of the life of these eggs in acid solution is a consequence of reduced metabolism. Increasing acidity up to a certain point leads, perhaps by an internal action or by a reversible alteration in the cortex which decreases the facility of interchange, to decreased metabolic activity; excessive acidity on the other hand produces irreversible injuries in the egg; where reduced metabolism and acid injury strike a reversible mean, the egg retains its viability for the longest time. The agreement between the pH at which maturation of *Asterias* eggs is completely inhibited and the pH of maximum viability conforms with this suggestion. The H-ion concentration of maximum viability may have some significance in relation to ovarian life, for in the ovary the egg is subjected to a greater CO_2 tension and H-ion concentration than that of sea water. But the cœlomic

fluids of *Asterias* and *Arbacia* are approximately neutral and it is unlikely that at any time the egg would naturally be subjected to an acidity so great as pH 6.0.

Summary.

A method is described for preparation of CO_2-free sea water of H-ion concentrations from pH 4.5 to 10.2.

The eggs of *Asterias, Arbacia* and *Chætopterus* retain for the longest time their capacity to fertilize and to divide at about pH 6.0.

Approximately this same H-ion concentration is required to completely repress the maturation process in *Asterias* eggs.

Chætopterus eggs are activated by exposures of 30 minutes or more to solutions of pH 5.0 to 6.6. If left in these solutions they show marked ameboid movements and fragmentation, but do not divide. If returned to sea water half or more of the eggs will undergo one or two abortive divisions. The activating effect of the acid sea water is most intense at about pH 5.8 to 6.0.

An exposure of 5 or 10 minutes to solutions of pH 5.0 to 6.6 (which is insufficient to activate) creates a block to fertilization which is permanent if the eggs are returned to sea water. If left from 30 minutes to several hours in the acid sea water, these eggs gradually recover their fertilizability, and when inseminated develop almost normally.

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